CANCER CELL-SPECIFIC OLIGOPEPTIDE SELECTED BY MICROFLUIDIC SYSTEM FROM A PHAGE DISPLAY LIBRARY FOR OVARIAN CANCER DIAGNOSIS

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ABSTRACT

Ovarian cancer has been one of the leading causes of death for women. However, it is still very challenging for its early diagnosis. The tedious conventional methods for screening specific biomarkers need at least two weeks or more. It was reported that cancer-specific oligopeptides could be screened from a phage display library. In this study, a new integrated microfluidic chip was developed to perform the entire screening process for specific oligopeptide selection of ovarian cancer cells. The screened oligopeptide was demonstrated to have high affinity with ovarian cancer cells and relatively low capture rate with other types of cancer cells. It is therefore promising to use the screened oligopeptide for isolation of ovarian cancer cells in the near future.

KEYWORDS: Ovarian cancer, Microfluidic, Phage display library, Biomarker

INTRODUCTION

Most ovarian cancer was caused from the epithelial or germ cell tumors of ovary. The age of women, gene mutation and status of obesity, pregnancy and endometriosis were major risk factors to affect the development of ovarian cancer. In 2010, approximately 160,000 people were dead from ovarian cancer. The disease was commonly observed in industrialized countries [1]. The clinical treatments of ovarian cancer usually involved chemotherapy, surgery and radiotherapy [2]. The 5-year survival rate was approximately 92% for early stage of ovarian cancer. However, it is challenging to diagnose it since its clinical symptoms are not significant. Unfortunately, 5-year survival rate of most patients who are diagnosed within the advanced stage is only 30%. It was well known to use the CA-125 biomarker, pelvic examination or transvaginal ultrasound for ovarian cancer diagnosis. However, it is still very challenging for its early diagnosis. Therefore, a new biomarker for early detection of ovarian cancer is critical to significantly improve clinical treatment.

It was reported that cancer-specific oligopeptides could be screened from a phage display library. The M13-based phage display library was successfully used to screen specific peptides targeting cellular receptors [3]. The identified oligopeptides therefore may pose clinical potential for rapid diagnosis or anti-cancer drug delivery in cancer therapy. However, tedious conventional methods for screening the oligopeptides need at least two weeks for specific biomarker selection. In this study, a new microfluidic chip was therefore developed to perform the entire screening process for specific oligopeptide selection for ovarian cancer cells. All of selection processes was automated in the device.

EXPERIMENTAL

Figure 1 schematically illustrates the strategy for screening of the cancer cell-specific oligopeptide that could recognize ovarian cancer cells (BG1) from a M13 phage display library on the microfluidic device. Briefly, phage display libraries and BG1 cells were first co-incubated at room temperature for 30 min. Next, Dynabeads® epithelial-enrich magnetic beads were used to capture the BG1 cells and collected by applying a magnetic field. Double-distilled water was then used as washing buffer to remove cell debris and free un-captured phage. Afterwards, *E. coli.* (ER2738) was added to co-culture with the phage-BG1 cell complex in Luria-Bertani (LB) broth. The cells swelled and were lyzed in bacterial culture medium. The DNA of bond M13-based phages then entered the cytoplasm, multiplied, assembled and secreted as mature phages from *E. coli*. After this "panning" process, the phages specific for BG1 cells were enriched by repeated rounds of panning and then the budding phage from *E. coli* was again collected in LB broth. The insert sequence of collected phages was then amplified, cloned and sequenced. Note that the whole panning time in the microfluidic device was significantly decreased (three times less) when compared with the conventional experiments.

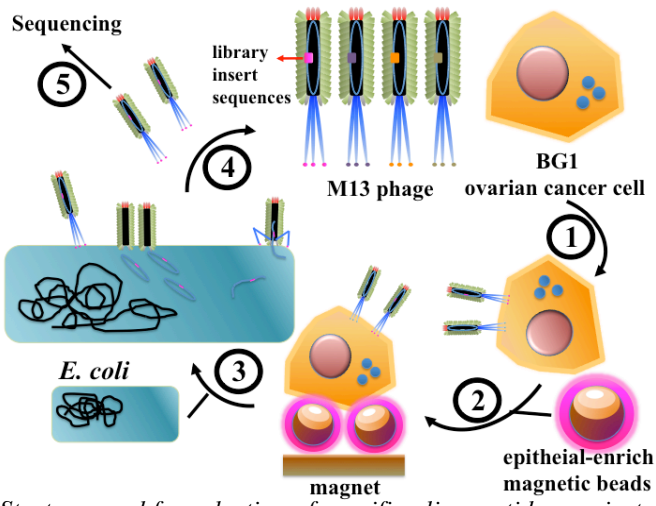
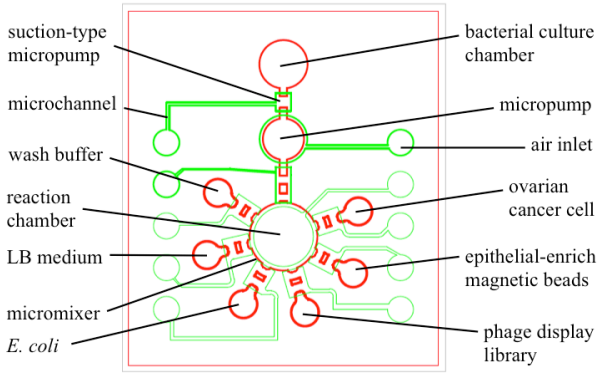


Figure 1: Strategy used for selection of specific oligopeptides against ovarian cancer cells from a phage display library on a microfluidic system. Step 1: phage library was incubated with ovarian cancer cells (BG1). Step 2: epithelial-enrich magnetic beads were used to capture BG1 cells and they were collected afterwards. Step 3: Free un-captured phage was removed by washing buffer and the phage-cell complex was cultured with E. coli. Step 4: screened phage was multiplied in bacterial cytoplasm and budding from E. coli. Step 5: After five panning selections, the selected phage was collected, cloned and sequenced.

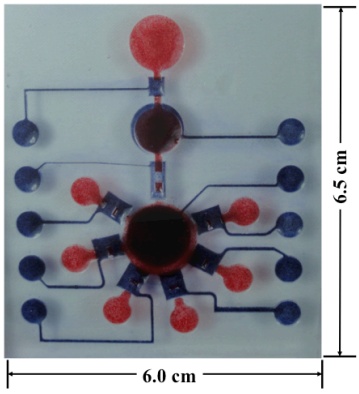
RESULTS AND DISCUSSION

An integrated microfluidic device was developed to perform the above-mentioned process. Figure 2(a) depicts a schematic diagram of the integrated microfluidic chip composed of microchambers, a micropump, microchannels and microvalves. The dimensions of the chip were measured to be 6.5 cm x 6.0 cm (Fig. 2(b)). The relationship between the pumping rate of the micropump and the operating air pressures was shown in Figure 2(c). All of insert sequences from collected phages were sequenced and classified to 24 groups.

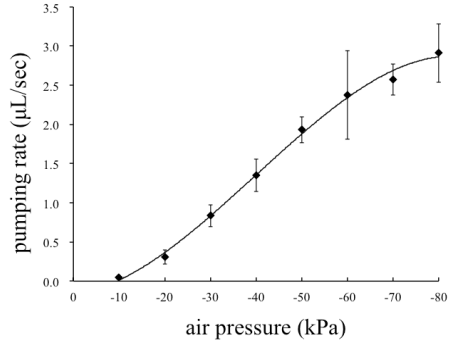
(a)



(b)



(c)



In this study, an oligopeptide was selected from the major group (15% of the whole sequences). The novel oligopeptide was sequenced, synthesized and conjugated onto the carboxylic magnetic beads. Figure 3 shows microscopic observations when the oligopeptide-coated beads were incubated with BG1 cells. The collected BG1 captured-oligopeptide–magnetic beads complex was then used to determine the capture rate of oligopeptide with BG1

cells. The un-coated beads could not capture any BG1 cells, as expected. Importantly, magnetic beads surface-coated with different concentrations of screened oligopeptides could successfully capture ovarian cancer cells BG1. Note that coated beads containing 10 pg of oligopeptide was sufficient to capture 10^5 BG1 cells by using homacytometer counting.

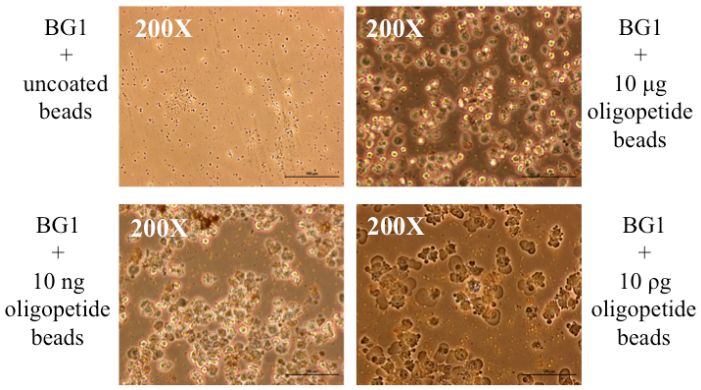


Figure 3: Microscopic photographs showed that magnetic beads surface-coated with different concentrations of screened oligopeptides could successfully capture ovarian cancer cells BG1.

The specificity and affinity of the screened oligopeptide was also confirmed by using flow cytometric analysis. 10 ng of FITC-labeled specific oligopeptide was used in this case. The flow cytometric results of capture rate testing for BG1 were similar to the one obtained from the oligopeptide-coated beads. The results demonstrated that the screened oligopeptide has high affinity (80.38%) with ovarian cancer cells. Conversely, the oligopeptide has a relatively low capture rate for other types of cancer cells, as listed in Table 1. Note that the capture rates of the specific oligopeptide against other cell lines were measured to be about 20%. This results are superior to the conventional antibody biomarkers [4]. It is then concluded that the new microfluidic device is well suited to screen and select highly specific oligopeptides against ovarian cancer cells in a rapid and automatic format.

Table 1. Capture rates of the screened oligopeptide for different cell types

tested cell	cell type	capture rate (%)
BG1	ovarian cancer	80.38±2.69
BxPC3	pancreatic cancer	12.89±0.21
HCT8	colorectal cancer	22.37±1.89
HeLa	cervical cancer	26.02±2.01
HepG2	liver cancer	15.77±5.25

CONCLUSION

A new integrated microfluidic system that involved with a phage display library method was developed for novel cancer biomarker screening. A novel oligopeptide was selected from the developed microfluidic device that recognized the ovarian cancer cell, BG1. The specific oligopeptide has high capture rate (about 80%) against BG1 cell and low capture rate (about 20%) for other cancer cell lines. The selected specific oligopeptide may be promising for ovarian cancer diagnosis in the near future.

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